EXHIBIT B

Therapeutic Drug Monitoring
20:467–477 © 1998 Lippincott Williams & Wilkins, Philadelphia

Pharmacodynamics, Pharmacokinetics, and Therapeutic Drug Monitoring of Glycopeptides

Alasdair P. MacGowan

Bristol Centre for Antimicrobial Research and Evaluation, Southmead Health Services, NHS Trust; and University of Bristol, Department of Medical Microbiology, Southmead Hospital, Westbury-on-Trym, United Kingdom

Summary: The glycopeptide antibacterial drugs, vancomycin and teicoplanin, are widely used in hospitals for therapy of severe or multiresistant infection that has a positive results on Gram's stain test. Although vancomycin resistance is common in some hospital-acquired *Enterococcus* sp and resistance to teicoplanin occurs among *Staphylococci* sp glycopeptides remain the cornerstone of therapy for infection due to methicillin-resistant *Staphylococcus aureus* (MRSA), coagulase-negative *Staphylococcus* organisms, and infection related to implanted devices. Therapeutic drug monitoring (TDM) of these agents remains controversial, but advances in our understanding of their pharmacodynamics and further clinical studies are helping clarify the situation. In the future, a more rational approach to monitoring will probably result in less intensive monitoring of vancomycin but more intensive monitoring of teicoplanin. Key Words: Vancomycin—Teicoplanin—Pharmacodynamics—Therapeutic drug monitoring.

Pharmacodynamics offers an opportunity to relate knowledge about an antimicrobial drug's in-vitro susceptibility, minimum inhibitory concentration (MIC), postantibiotic effect (PAE), pattern of bactericidal action, and interactions with immune cells to pharmacokinetics to optimize drug dosing regimens.

Postantibiotic effect is the ability of an antibacterial to suppress the generation of bacteria for several hours after antibacterial concentrations have fallen below the MIC. Although the exact mechanism of the PAE is unknown, it may be related to repair of damaged, but not killed, cells; separation of bound drug from target; or synthesis of new enzymes or proteins (1). Penicillins, cephalosporins, macrolides, and aminoglycosides have a PAE against bacteria that have a positive result on Gram's stain tests (2). Although the measurement of the PAE depends on the method used (3), vancomycin has been shown by a number of techniques to have a PAE of 2 to 3 hours against *Staphylococcus aureus* (2,4,5).

Teicoplanin also has a PAE that appears to be longer than that of vancomycin (6,7). If a concentration well below the MIC is allowed to remain instead of completely removing the antibiotic during the measurement of the PAE, the PAE duration is doubled for vancomycin, which is termed a sub-MIC effect. This measure is akin to the exponential decay of a drug concentration in serum (8).

Increasing the concentration of vancomycin in the therapeutic range (i.e., from 5 mg/L to 40 mg/L) does not increase the time to kill 99.9% of the bacterial population or the rate of kill (9); the rate of killing is slower for teicoplanin than for vancomycin, perhaps because of the former's high protein binding (10).

The pharmacodynamics of glycopeptides studied in several animal models support the concept that high initial concentrations offer no advantage in bacterial killing or mortality, whereas higher, sustained concentrations or more frequent dosing have improved survival in animal models of infective endocarditis (11,12).

In a complex analysis using a mouse model, multiple

Received February 11, 1998; accepted July 23, 1998.

Address correspondence and reprint requests to Alasdair P. MacGowan, University of Bristol, Department of Medical Microbiology, Southmead Hospital, Westbury-on-Trym, Bristol BS10 5NB United Kingdom.

pharmacodynamic parameters were compared with the effective dose $_{50}$ (ED_{50}). The time serum concentration (T) exceeded the MIC (T > MIC) and was best related to ED_{50} when treating penicillin-resistant *Pneumococci* organisms with either vancomycin or teicoplanin (13). Use of an in vitro, continuous bacterial culture model supports this finding. By simulating four different therapeutic regimens with various peak or trough concentrations or areas under the curve (AUC), but maintaining T > MIC at 100%, it was shown that there were no differences in the degree or rate of *S. aureus* killing (14).

Laboratory and animal evaluation of glycopeptide pharmacodynamics indicates that glycopeptides do not show concentration-dependent killing in the therapeutic range; hence, high postdose concentrations are unlikely to be of benefit. In addition, they have a PAE and sub-MIC effects, indicating that serum concentrations need not exceed the MIC for all of the dosing interval and that T > MIC or sustained concentrations are related to outcome. Protein binding affects bacterial killing with teicoplanin. Therefore, the dosing interval is probably best optimized as T > MIC plus PAE, although clinically, with conventional doses of vancomycin, T > MIC is 100%. This result had led some to propose that smaller doses than the standard 2 grams per day of vancomycin may be just as effective in clinical practice; alternatively, longer dosing intervals may be appropriate for glycopeptides (15).

PHARMACOKINETICS

The pharmacokinetics of vancomycin and teicoplanin have been extensively studied and are known to vary in different patient groups. For example, vancomycin handling is changed in renal impairment (16–18); obesity (19,20), liver failure (21); various renal support therapies (22–27), neutropenia (28), malignancy (29), age, and gender (30); and with sepsis and its therapy (Table 1 [31,32]).

Similarly, teicoplanin pharmacokinetics are altered in renal impairment (33,34), renal support therapies (35,36); children and the elderly (37), intravenous (IV) drug abusers (38), burn patients (9), and neutropenia (see Table 1 [40]). In addition, it is clear that standard dosing of teicoplanin (400 mg × 2 for 24 hours, then 400 mg 24 hourly) results in significant numbers of patients having predose serum concentrations of 10 mg/L (40).

However, pharmacokinetic variability on its own can rarely be a justification for TDM and only becomes important if serum concentrations can be linked to toxicity or efficacy. This link has been tenuous for glycopeptides and continued TDM was questioned in the late 1980s and early 1990s (42–46). This process has now resulted in the emergence of new data.

TABLE 1. *Patient factors affecting vancomycin or teicoplanin pharmacokinetics*

Patient Factor	Pharmacokinetic change	Reference no.
Vancomycin		
Renal impairment	Increasing t½ with decreasing creatinine clearance	17
Chronic intermittent hemodialysis	As for renal impairment: little drug removed by dialysis	22
Chronic intermittent peritoneal dialysis	Prolonged t½	23
Continuous veno–venous hemofiltration or dialfiltration	Increased clearance compared to hemodialysis	25,27
Obesity	Shorter t½, larger volume of distribution	18,19
Liver failure	Longer t½	21
Age	Longer t½ in neonates than infant and children	29,36
Sepsis	Prolonged t½	25
Teicoplanin		
Renal impairment	Increasing t½ with decline in creatinine clearance	33,34
Chronic intermittent hemodialysis	Reduced clearance	35
Chronic ambulatory peritoneal dialysis	Prolonged t½, increased volume of distribution	52
Continuous veno–venous hemofiltration	Prolonged t½	35
Continuous hemofiltration	t½ not prolonged	36
Intravenous drug abusers	Increased clearance	38
Burn patients	Increased t½	39
Neutropenia	Increased elimination, larger interindividual variability	40

TOXICITY

Vancomycin serum monitoring, if performed, is aimed at reducing the risks of nephrotoxicity or ototoxicity; it will not reduce immediate or infusion-related toxicities. Ototoxicity is difficult to assess clinically and data is sketchy because they are often composed of case reports in patients with renal failure, who sometimes have high serum concentrations. It is not sufficient to make any association between concentrations and toxicity.

The incidence of nephrotoxicity is probably less than 5% in patients treated with vancomycin alone, but higher if a combination of vancomycin plus an aminoglycoside is used (47). Toxicity is also associated with longer courses of therapy and the original report of Faber and Moellering (47) linked three patients to trough concentrations of 30 mg/L to 65 mg/L before the onset of tox-

icity. There are now several reports that vancomycin TDM services involving prescription review, blood concentration measurement, and dose modification by clinical pharmacists can reduce the incidence of nephrotoxicity. In a 1994 prospective cohort study in a teaching hospital, 116 patients who received more than 4 days' therapy and were not neutropenic or in an intensive care unit or established renal failure were studied. It was shown that the rate of nephrotoxicity was 24% in patients not randomized to the TDM service, compared with 7% of those who received TDM (48). A similar and prospective randomized study in 70 patients with hematologic malignancy indicated that nephrotoxicity was lower in patients recruited into the TDM arm (mild toxicity, 13.5%; moderate, 0%) compared with those who received no TDM (mild, 33%; moderate, 9.1% [49]). Furthermore, in a retrospective review of 273 patients with positive results of infection with Gram's stain, it was shown that serum vancomycin concentrations before onset of nephrotoxicity were higher (23.5 [2.5 mg/L]) in those in whom toxicity developed than in those in whom it did not (10.2 [3.8 mg/L] [50]). In contrast, in a prospective study of patients randomized to have dose adjustment to achieve predose concentrations in the ranges of 5 mg/L to 10 mg/L, 10 mg/L to 15 mg/L, or 15 mg/L to 25 mg/L, no correlation was found to nephrotoxicity (51).

Thrombocytopenia associated with large doses of teicoplanin (30 mg/kg per day) has recently been related to trough concentrations; for those with trough concentrations of more than 60 mg/L, eight of 58 patients had a decrease in platelets, whereas with trough concentrations of less than 60 mg/L, 12 of 251 had a decrease in platelets ($p < 0.05$) [52]).

OUTCOME

Retrospective data reviews of teicoplanin clinical trials have indicated that serum concentrations are related to clinical outcomes. In an open multicenter study in which most patients had right-sided infection due to *S. aureus* and predose concentrations had been adjusted to between 10 mg/L to 15 mg/L, it was reported that postdose concentrations of teicoplanin of more than 40 mg/L were associated with improved outcome (53). A further study (mainly of bone infection due to *S. aureus*) suggested that larger doses than were conventionally used at that time were required for successful therapy and that average troughs were 36.3 ($n = 10$) in those successfully treated, and only 9.7 mg/L in the three clinical failures (54). A retrospective review of three trials in the United States indicated an association between increased dose, high trough serum levels, trough concentration/MIC ratio and days to clear bacteremia, fever days, and clinical improvement (55). A further retrospective review of 58 cases published with sufficient pharmacokinetic and susceptibility data for analysis indicated a relationship between predose and postdose teicoplanin concentrations and predose/MIC or postdose/MIC ratios and clinical outcomes. Dose was not related to outcome in this review, in which most of the patients had severe staphylococcal infection (56). In a more recent review of 92 patients with *S. aureus* bacteremia using a multivariate analysis to relate age, weight, dose, loading dose, combination therapy, and serum concentrations to outcome has shown that only trough concentration and age were significantly related to outcome (57). A prospective study of teicoplanin to treat *S. aureus* infective endocarditis showed that if the predose concentration was less than 20 mg/L, six of 10 patients failed, compared with one of 11 if the concentration was more than 20 mg/L ($p < 0.05$) (58).

The data relating to vancomycin serum concentration to efficacy is less clear. Two prospective studies showed that therapeutic drug monitoring (TDM) services had no effect on efficacy (48,49) and an intervention in which patients were deliberately stratified into three groups with predose targets of 5 mg/L to 10 mg/L, 10 mg/L to 15 mg/L, or 15 mg/L to 25 mg/L showed no difference in fever days or clinical outcome (51). In contrast, two retrospective reviews were able to relate serum concentrations to outcome measures. In a retrospective review of 273 patients with positive results of infection proven with Gram's stain, Zimmerman and colleagues (50) were able to relate troughs of more than 10 mg/L to a reduced number of fever days and an improved white blood cell response but not to lengths of stay or mortality. Mulhern and colleagues (59) related trough concentrations to relapse rates in patients with peritonitis who were treated with continuous ambulatory peritoneal dialysis for end-stage renal disease. When the mean predose concentration was less than 12 mg/L, 9 of 14 patients relapsed; when it was more than 12 mg/L, none of 17 relapsed.

In conclusion, pharmacodynamic principles indicate that predose glycopeptides should be related to the outcome of infection measures. Evidence now exists in humans based on teicoplanin therapy of staphylococcal infections; the evidence is less conclusive for vancomycin.

For both vancomycin and teicoplanin, there is data to link predose concentrations to toxicity (nephrotoxicity for vancomycin and thrombocytopenia for teicoplanin). Table 2 summarizes present recommendations for glycopeptide TDM, including those which have been used and criticized in the past, and more streamlined recommendations that may be more appropriate for the future.

TABLE 2. *Recommendations for therapeutic drug monitoring glycopeptides*

	Patient group	Serum concentrations
Vancomycin	All patients on therapy >4 to 5 days (>2 days if conservative)	Predose only, mg/L 5–15 mg/L; Predose, 5–10 mg/L; postdose 20–40 mg/L, if conservative
Teicoplanin	Severe infection *Staphylococcus aureus* severe infection *Staphylococcus aureus* IE Other IE IVDA Renal impairment Maintain teicoplanin troughs at <50 mg/L	Predose, >10 mg/L Predose, >10 mg/L (>20 mg/L if conservative) Predose, >20 mg/L Predose, >10 mg/L; postdose, >40 mg/L

IE, infective endocarditis; IVDA, intravenous drug abuser.

REFERENCES

1. Gould IM. Pharmacodynamics and the relationship between in vitro and in vivo activity of antimicrobial agents. *J Chemother* 1997;9:74–83.
2. Bundtzen RW, Gerber AU, Holm DL, et al. Post antibiotic suppression of bacterial growth. *Rev Infect Dis* 1981;3:28–37.
3. MacKenzie FM, Gould IM. The post antibiotic effect. *J Antimicrob Chemother* 1993;32:519–37.
4. Hunberger H, Nikuni I-E, Mihano M, et al. Post antibiotic effect of beta lactams antibiotics on Gram-negative bacteria in relation to morphology, initial killing and MIC. *Eur J Clin Microbiol Infect Dis* 1991;10:927–34.
5. Hunberger H. Pharmacodynamic effects of antibiotics: Studies on bacterial morphology, initial killing, post antibiotic effect and effective regrowth time. *Scand J Infect Dis* 1992;81:3–52.
6. Tanaka K, Kuchi K, Shimizu K, et al. Post antibiotic effect and clinical evaluation of teicoplanin. *Nippon Ganyaku Ryoho Gakki Zasshi* 1993;41:373–7.
7. Cooper MA, Tin YF, Addey JF, et al. In vitro comparison of the post-antibiotic effect of vancomycin and teicoplanin. *J Antimicrob Chemother* 1990;26:203–7.
8. Odenholt-Tornqvist I, Lowdin E, Cars O. Post antibiotic sub-MIC effects of vancomycin, roxithromycin, sparfloxacin and amikacin. *Antimicrob Agents Chemother* 1992;36:1832–8.
9. Lacroix AJ, Walker RJ, Redden JM, et al. The concentration-independent effect of monoexponential and biexponential decay in vancomycin concentrations on the killing of *Staphylococcus aureus* under aerobic and anaerobic conditions. *J Antimicrob Chemother* 1996;38:589–97.
10. Bailey EM, Rybak MJ, Kaatz GW. Comparative effect of protein binding on the killing activities of teicoplanin and vancomycin. *Antimicrob Agents Chemother* 1991;35:1089–92.
11. Contopoulos A, Joly V, Abel L, et al. The pharmacokinetics and extravascular diffusion of teicoplanin in rabbits and comparative efficacy with vancomycin in an experimental endocarditis model. *J Antimicrob Chemother* 1988;21:621–31.
12. Chambers HF, Kennedy S. Effect of dosage, peak and trough concentrations in serum, protein binding and bactericidal rate on efficacy of teicoplanin in a rabbit model with endocarditis. *Antimicrob Agents Chemother* 1990;34:510–4.
13. Knudsen JD, Fuursted K, Espersen F, et al. Activities of vancomycin and teicoplanin against penicillin-resistant pneumococci in vitro and in vivo and correlation to pharmacokinetic parameters in the mouse peritonitis model. *Antimicrob Agents Chemother* 1997; 41:1910–5.
14. Daffet SB, Begg EJ, Chambers ST, et al. Efficacies of different vancomycin dosing regimens against *Staphylococcus aureus* determined with dynamic in vitro model. *Antimicrob Agents Chemother* 1994;38:2680–2.
15. MacGowan AP, Bowker KE. Pharmacodynamics of antimicrobial agents and rationale for their dosing. *J Chemother* 1997;9:64–73.
16. Moellering RC, Krogstad DJ, Greenblatt DJ. Pharmacokinetics of vancomycin in normal subjects and in patients with reduced renal function. *Rev Infect Dis* 1981;3:229–34.
17. Matzke GR, McGory RW, Halstenson CE, et al. Pharmacokinetics of vancomycin in patients with various degrees of renal function. *Antimicrob Agents Chemother* 1984;25:433–7.
18. Cunha BA, Quintiliani R, Deglin JH, et al. Pharmacokinetics of vancomycin in anuria. *Rev Infect Dis* 1981;3:229–72.
19. Blouin RA, Bauer LA, Miller DD, et al. Vancomycin pharmacokinetics in normal and morbidly obese subjects. *Antimicrob Agents Chemother* 1982;21:575–80.
20. Vance-Bryan K, Guay DRP, Gilliland SS, et al. Effect of obesity on vancomycin pharmacokinetic parameters as determined by using a Bayesian forecasting technique. *Antimicrob Agents Chemother* 1993;37:436–40.
21. Brown N, Ho DHW, Fong KLL, et al. Effect of hepatic function on vancomycin clinical pharmacology. *Antimicrob Agents Chemother* 1983;23:680–9.
22. Magora RE, Arroyo JC, Rusansky SJ, et al. Vancomycin pharmacokinetics in patients with peritonitis on peritoneal dialysis. *Antimicrob Agents Chemother* 1983;23:710–4.
23. Blouin RD, Halstenson CE, Salem NO, et al. Pharmacokinetics of vancomycin in patients undergoing continuous ambulatory peritoneal dialysis. *Antimicrob Agents Chemother* 1984;25:603–6.
24. Touchais MA, Patel RV, Anumba JV, et al. Vancomycin removal by high-flux polysulfone haemodialysis membranes in critically ill patients with end-stage renal disease. *Am J Kidney Dis* 1995;26: 469–74.
25. Ifrensolle F, Kinowski JM, de la Coussaye JE, et al. Clinical pharmacokinetics during continuous haemofiltration. *Clin Pharmacokinet* 1994;26:457–71.
26. Alwohell J, Najjar TA, el Yazeji MJ, et al. Comparison of the effects of three haemodialysis membranes on vancomycin disposition. *Int Urol Nephrol* 1994;26:223–8.
27. Sentre C, Lavoy G, Simpo M, et al. Pharmacokinetics of vancomycin during continuous haemodiafiltration. *Intensive Care Med* 1993;19:347–50.
28. Le Normand Y, Milpied N, Kergueris MF, et al. Pharmacokinetic parameters of vancomycin for therapeutic regimens in neutropenic adult patients. *Int J Biomed Comput* 1994;36:121–5.
29. Chang D. Influence of malignancy on the pharmacokinetics of vancomycin in infants and children. *Pediatr Infect Dis J* 1995;14: 667–79.
30. Seay RE, Brundage RC, Jensen PD, et al. Population pharmacokinetics of vancomycin in neonates. *Clin Pharmacol Ther* 1994;56: 169–75.
31. Rotschafer JC, Crossley K, Zaske D, et al. Pharmacokinetics of vancomycin: Observations in 28 patients and dosage recommendations. *Antimicrob Agents Chemother* 1982;22:391–4
32. Pou L, Rosell M, Lopez R, et al. Changes in vancomycin pharmacokinetics during treatment. *Ther Drug Monit* 1996;18:149–53.
33. Stahlmann JA, Thompson GA, Kenny MT, et al. Applicability of teicoplanin dosage adjustment guidelines for renally impaired patients over the range 3 to 30 mg/kg^{-1}. *Biopharm Drug Dispos* 1992;13:371–91.
34. Derbyshire N, Webb DB, Roberts D, et al. Pharmacokinetics of teicoplanin in subjects with varying degrees of renal function. *J Antimicrob Chemother* 1989;23:869–73.
35. Hoffler DK, Jaeppe P, Naumann E, et al. Pharmacokinetics of teicoplanin haemodialysis patients. *Infection* 1991;19:324–7.
36. Hillaire-Buys D, Peytavi H, Lobjois P, et al. Influence of arteriovenous haemofiltration on teicoplanin elimination. *Br J Clin Pharmacol* 1995;40:95–7.

37. Kaner N, Dubos JP, Roussat-Devallez M, et al. Teicoplanin and amikacin in neonates with staphylococcal infection. *Pediatr Infect Dis J* 1993;12:510–3.
38. Rybak MJ, Lerner SA, Levine DP, et al. Teicoplanin pharmacokinetics in intravenous drug abusers being treated for bacterial endocarditis. *Antimicrob Agents Chemother* 1991;35:696–700.
39. Steer J, Pupini R, Wilson APR, et al. Pharmacokinetics of a single dose of teicoplanin in burn patients. *J Antimicrob Chemother* 1996; 47:545–53.
40. Lortholary O, Tod M, Rizzo N, et al. Population pharmacokinetic study of teicoplanin in severely neutropenic patients. *Antimicrob Agents Chemother* 1996;40:1263–7.
41. MacGowan AP, McMullin CM, White LO, et al. Serum monitoring teicoplanin. *J Antimicrob Chemother* 1992;30:399–402.
42. Edwards DI, Pancorbo S. Routine monitoring of serum vancomycin concentrations: Waiting for proof of its value. *Clin Pharmacol* 1987;6:652–4.
43. Rodvold KA, Zaluka H, Rotschafer JC. Routine monitoring of serum vancomycin concentrations: Can waiting be justified? *Clin Pharmacol* 1987;6:655–8.
44. Freeman CD, Quintiliani R, Nightingale CH. Vancomycin therapeutic drug monitoring: Is it necessary? *Ann Pharmacol* 1993;27: 594–8.
45. Canu TO, Yamaneta-Yuco Na, Lehman PS. Serum vancomycin concentrations: Reappraisal of their clinical value *Clin Infect Dis* 1994;18:533–43.
46. Moellering RC. Editorial: Monitoring serum vancomycin levels: Climbing the mountain because it is there. *Clin Infect Dis* 1994; 18:544–6.
47. Faber BP, Moellering RC. Retrospective study of the toxicity of preparations of vancomycin from 1974 to 1981. *Antimicrob Agents Chemother* 1983;23:138–41.
48. Welty TE, Copa AK. Impact of vancomycin therapeutic drug monitoring on patient care. *Ann Pharmacother* 1994;28:1335–9.
49. Fernandez de Gatta MD, Calvo MV, Hernandez JM, et al. Cost effectiveness analysis of serum vancomycin concentration monitoring in patients with haematologic malignancy. *Clin Pharmacol Ther* 1996;60:332–40.
50. Zimmerman AE, Kamen BG, Plaisance KI. Association of vancomycin serum concentrations with outcome in patients with Gram-positive bacteremia. *Pharmacotherapy* 1995;15:85–91.
51. Rybak MJ, Cappelletty MJ, Ruffing RC, et al. Influence of vancomycin serum concentrations on the outcome of patients being treated for Gram-positive infections. *Abstr 37th Interscl Conf Antimicrob Agents Chemother* 1997;A46.
52. Wilson APR, Gruneberg RN. Safety. In: *Teicoplanin: The first decade*. Abingdon, UK: The Medicine Group 1997:137–44.
53. Lepori C, Perronne C, Massip P, et al. Evaluation of teicoplanin for treatment of endocarditis caused by Gram-positive cocci. *Antimicrob Agents Chemother* 1989;33: 871–6.
54. Gruneberg RN. Treatment of bone, joint and vascular-access-associated Gram-positive bacterial infections with teicoplanin. *Antimicrob Agents Chemother* 1990;33:2392–7.
55. Rybak MJ, Cappelletty DM, Kang SL, et al. Pharmaco-dynamic evaluation of teicoplanin versus vancomycin in the treatment of Gram-positive bacteremia and endocarditis. *Abstr 36th Interscl Conf Antimicrob Agents Chemother* 1996;A36.
56. MacGowan A, White L, Reeves D, et al. Retrospective review of serum teicoplanin concentrations in clinical trials and their relationship to clinical outcome. *J Infect Chemother* 1996;2:197–208.
57. MacGowan AP, White LO, Reeves DS, et al. Teicoplanin in Staphylococcus aureus septicaemia: Relationship between trough serum levels and outcome. *37th Interscl Conf Antimicrob Agents Chemother* 1997;A45.
58. Wilson APR, Gruneberg RN, Nau H. A critical review of the dosage of teicoplanin in Europe and the USA. *Int J Antimicrob Agents* 1994;4:51–30.
59. Mulhern JG, Beadle GL, O'Shea MH, et al. Trough serum vancomycin levels predict the relapse of gram-positive peritonitis in peritoneal dialysis patients. *Am J Kidney Dis* 1995;25:611–5.